Figure S1 related to figure 1

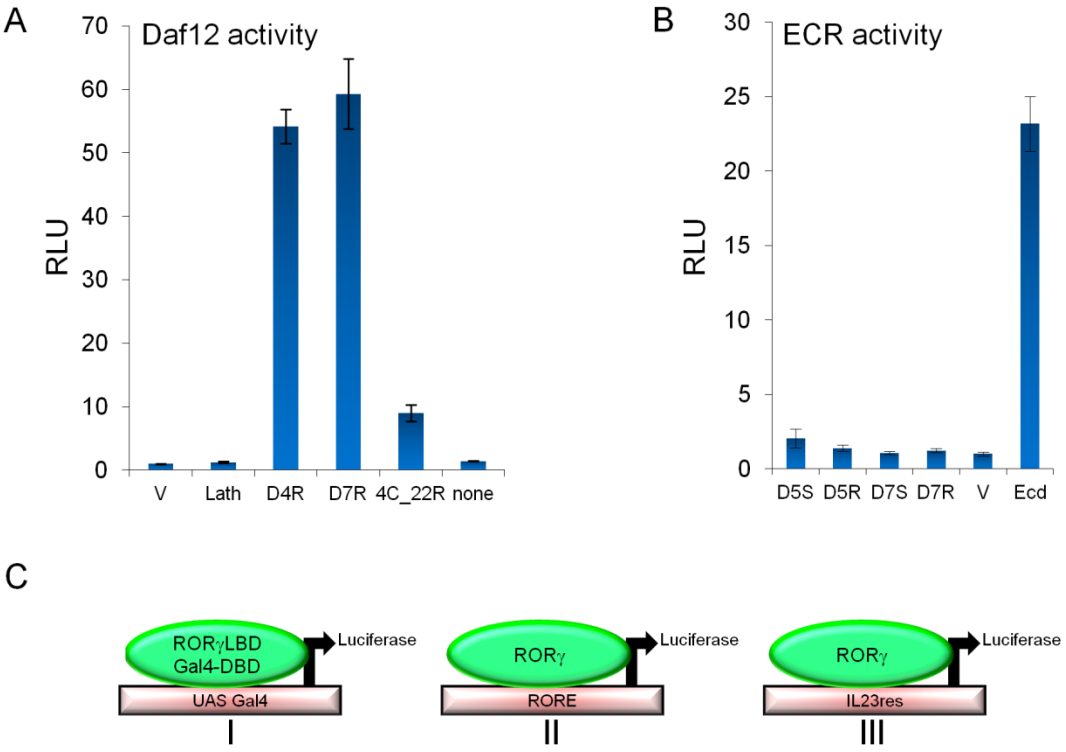


Figure S1 related to Figure 1. Reporter activities of NHRs for cognate ligands are not affected in insect cells grown in CDM conditions. (A). Reporter activity of Daf12-gal4/UAS firefly luciferase reporter in Kc167 cells maintained in SSM1 media. Vehicle (V), lathosterol (lath), Daf12 ligands Dafachronic acid delta 4R (D4R), dafachronic acid delta 7R (D7R) and 4-cholesten-22R hydroxyl (4C_22R) and no ligand (none). Representative experiments of n=2. (B) Reporter activity of ecdysone receptor (Ecr) ECR-gal4/UAS firefly luciferase reporter in Kc167 cells maintained in SSM1 media. Vehicle (V), Dafachronic acid delta 5S (D5S), delta 5R (D5R), delta 7S (D7S) and delta 7R (D7R). ecd is ecdysone. Representative experiment of n=3. Experiments are technical triplicates. Error bars are standard deviations. (C) Mammalian and insect reporter systems used in this study: I. RORγ-gal4 fusion protein driving a UASgal4 firefly luciferase reporter; II. Full length RORγ protein driving a ROR element (RORE) firefly luciferase reporter; III. A full length RORγ driving a natural IL23 responsive promoter based firefly luciferase reporter.

Figure S2 related to figure 2 and 3

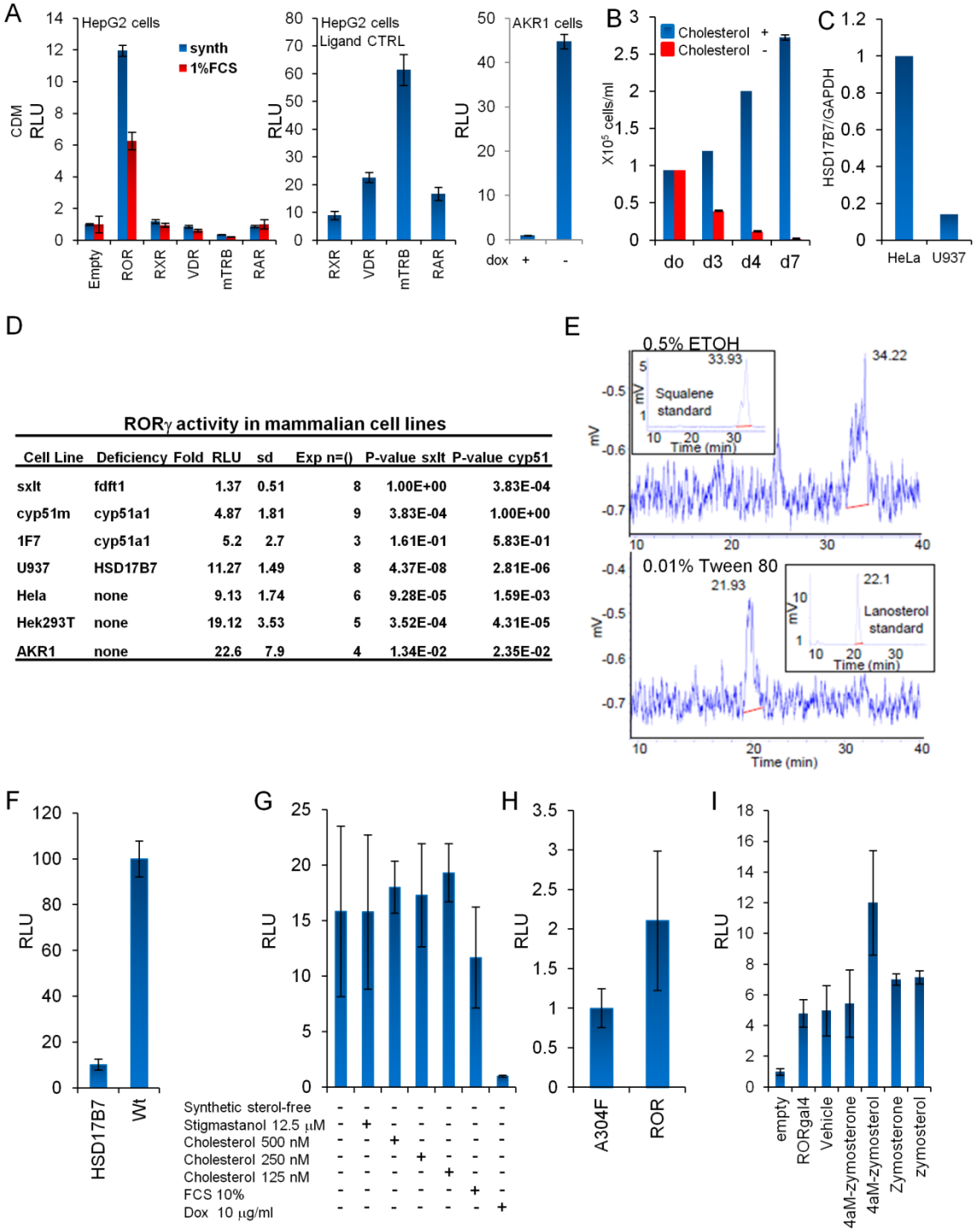


Figure S2 related to figure 2 and 3. RORγ is constitutively active in mammalian cells. (A) RORγ reporter activity is detected in mammalian cells. HepG2 cells were transiently transfected with RORγ-gal4/UAS firefly luciferase reporter CDM (Blue) bars and FCS media (red) bars. Positive controls for different NHRs (middle panel): RXR 9-cis-retinoic acid; VDR Vitamin D3; TRβ Triiodothyronine; RARα all-trans retinoic acid (ATRA). AKR1 cells (right panel) stably transfected with tTA-OFF inducible RORγ-gal4/UAS firefly luciferase reporter were maintained in CDM with or without 10 μg/ml doxycyclin. Representative experiment of n=2. (B) U937ROR cells are dependent on exogenous cholesterol. 1 x10^5 U937 cells were inoculated in basal serum-free media with or without synthetic cholesterol. Cells were checked for growth and viability at specific time intervals (d=day 0,3,4 and 7). (C) U937ROR cells have reduced ratio of mRNA for the enzyme *HSD17B7* when compared to HeLa cells. RNA was extracted from both cell lines and RT-PCR performed using *GAPDH* as a standard for comparison of *HSD17B7* ratios. (D). RORγ reporter activity in different mammalian cell lines maintained in DMEM media with 1% FCS. Columns are cell lines, specific cholesterol biosynthetic pathway deficiencies, average fold induction from RORγ-gal4 to empty vector control, standard deviation of fold induction averages and *P*-values comparing cell lines to SXLT or to *Cyp51*$^{-/-}$ cells. Statistics are two-tailed non-paired Student's *t*-test. (E) Squalene uptake and metabolization by fibroblasts (top). 0.5 μCi ^{3}H-squalene added as a 0.5% ethanol solution to the medium without serum. (Bottom) 0.5 μCi ^{3}H-squalene added in 0.01% Tween 80 emulsion to the medium without serum. Peak with Rt = 22 min could represent lanosterol (inlay) or a later cholesterol intermediate. Measured is the radioactive signal in mV. (F) Overexpression of *HSD17B7* in U937ROR reduces RORγ reporter activity. A clone of U937ROR cells was transfected with *HSD17B7* and selected in synthetic media without cholesterol for 7-14 days. Under these conditions only cells that recovered the ability to produce cholesterol survive and proliferate. Cells are then transferred RPMI-10% FCS media and luciferase reporter activity in these cells is compared to that of wild type cells of the same clone grown in RPMI-10% FCS media, n=2 experiments. (G). The RORγ reporter activity of U937ROR cells is not dependent on cholesterol. U937ROR cells were grown in synthetic media in the absence or presence of

cholesterol in concentrations required for cell proliferation and survival. N=2 experiments. Conversely, cells were maintained in 10% FCS. 10 μg/ml doxycyclin was used to inhibit expression of the RORγ reporter. (H) FF-MAS induces RORγ specific reporter activity in *Cyp51*$^{-/-}$ fibroblast 1F7. 1F7 cells were transfected and as described in C with either inactive RORγ mutant A304F or RORγgal4. After 24 hrs cells were incubated overnight with 10 μM FF-MAS and luciferase reporter activity measured. (I) 4a-methyl-zymosterol is a weak agonist for RORγ. HEK293T cells were transfected as described in A. n=2 experiments. Bar and lines are averages of technical triplicates. Error bars are standard deviations (SD).

Figure S3 to main text "Characterization of lipids bound to RORγ immunoprecipitated from mammalian cells"

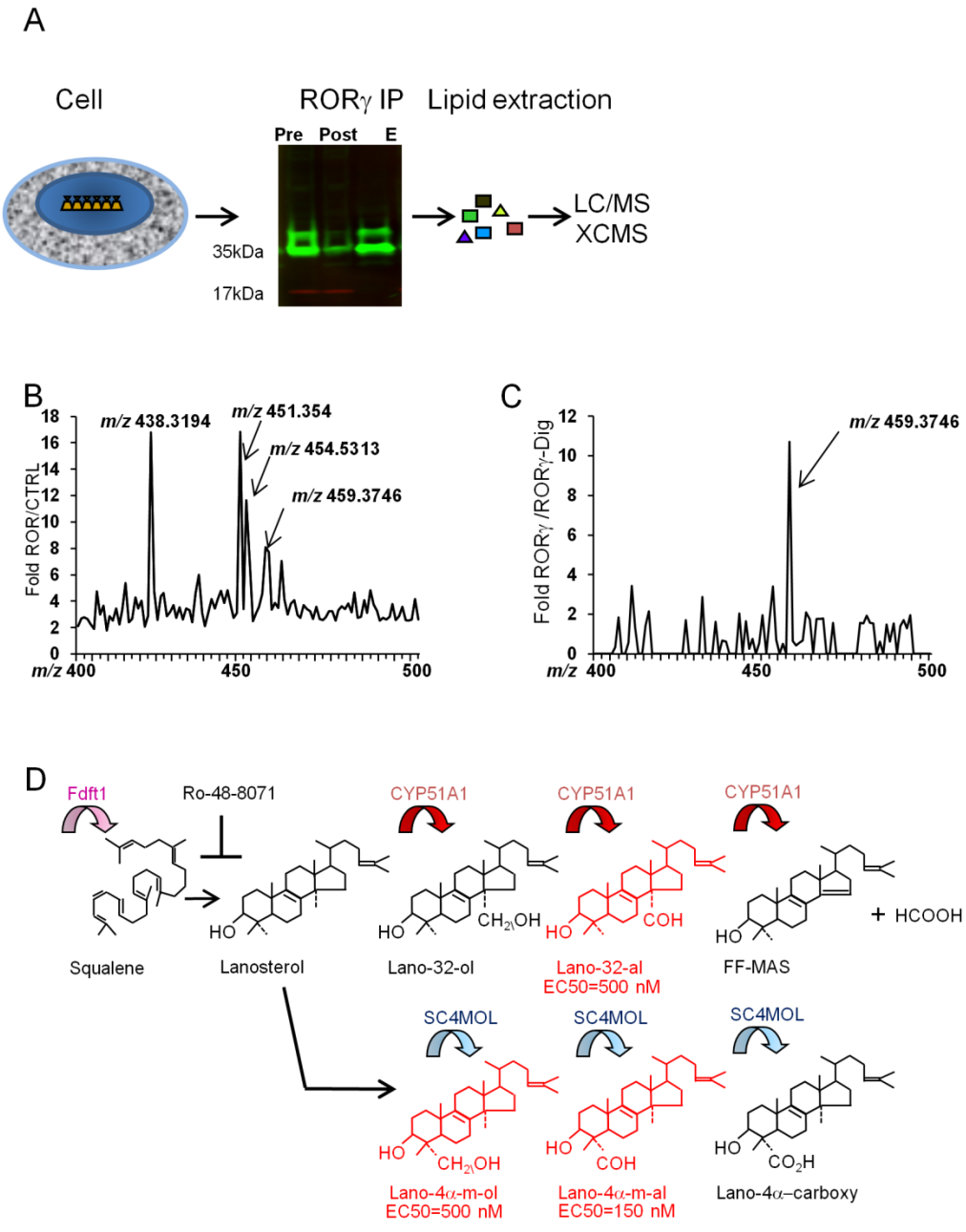


Figure S3 related to main text "Characterization of lipids bound to RORγ immunoprecipitated from mammalian cells". Derivatives of CBIs are immunoprecipitated with RORγ. (A) Flag-RORγ LBD was immunoprecipitated from nuclear extract of HEK293T cells with anti-FLAG antibody. Samples of the nuclear extract were collected for western blot analysis before (pre) and after (post) immunoprecipitation with anti-FLAG antibody, as well as after elution (E) with FLAG peptide. Flag-RORγ LBD (green) and loading control Histone H3 (red). The lipid in the immunoprecipitate was extracted, analyzed by LC-MS and data processed with XCMS package. (B) Set of major ions enriched in RORγ-LBD versus empty vector plasmids *(P< 0.0002). P-values* defined by two-tailed unpaired Student's *t*-test. (C) The *mz* 459.3746 $[M+H]^+$ ion was selectively depleted in RORγ-LBD positive nuclear extract treated with 30 μM RORγ inhibitor digoxin. Compared are independently prepared samples (biological replicates) of 4 digoxin-treated and 4 non-treated RORγ IPs. *P <0.00003. P-values* defined by two-tailed unpaired Student's *t*-test and accuracy of exact mass measurements (±0.0091) Da. (D) Metabolites of lanosterol can be grouped as canonical when they are derived from metabolism of lanosterol by CYP51A1 and non-canonical when derived from metabolism of lanosterol by SC4MOL. The metabolites tested in this study are marked in red. The metabolites of CYP51A1 are formed by modification of C32 which is the methyl group attached at C14 into lano-32-ol (14α–hydroxymethyl,4α,4β-dimethyl-cholesta-8,24-dien-3-ol) which can reach near 1% of total cellular sterol in hepatocytes (Trzaskos et al., 1987) and then Lano-32-al (14α–formyl,4α,4β-dimethyl-cholesta-8,24-dien-3-ol). The EC_{50} of Lanosterol-32-al in Kc167 cells was defined as described in Figure 1C and was calculated as EC_{50}=500 nM.

The non canonical derivatives of lanosterol occur when the C4-methyl group of Lanosterol is removed before the C14-methyl group giving origin to 4α–hydroxymethyl,4β,14α-dimethyl-cholesta-8,24-dien-3-ol (Lano-4α-ol) (EC_{50}=500 nM), 4α–formyl,4β,14α-dimethyl-cholesta-8,24-dien-3-ol (Lano-4α-al) (EC_{50}=150 nM) and 4α–carboxy,4β,14α-dimethyl-cholesta-8,24-dien-3-ol (Lano-4α-carboxy). The EC_{50} measurements for the non-canonical metabolites are from the Δ^7 analogues of lanosterol and for which a peak corresponding to 4α–hydroxymethyl,4β,14α-dimethyl-cholesta-7,24-dien-3-ol (Lano-4α-ol) was found in thymus at concentrations of 60 nM.

Figure S4 related to figure 4

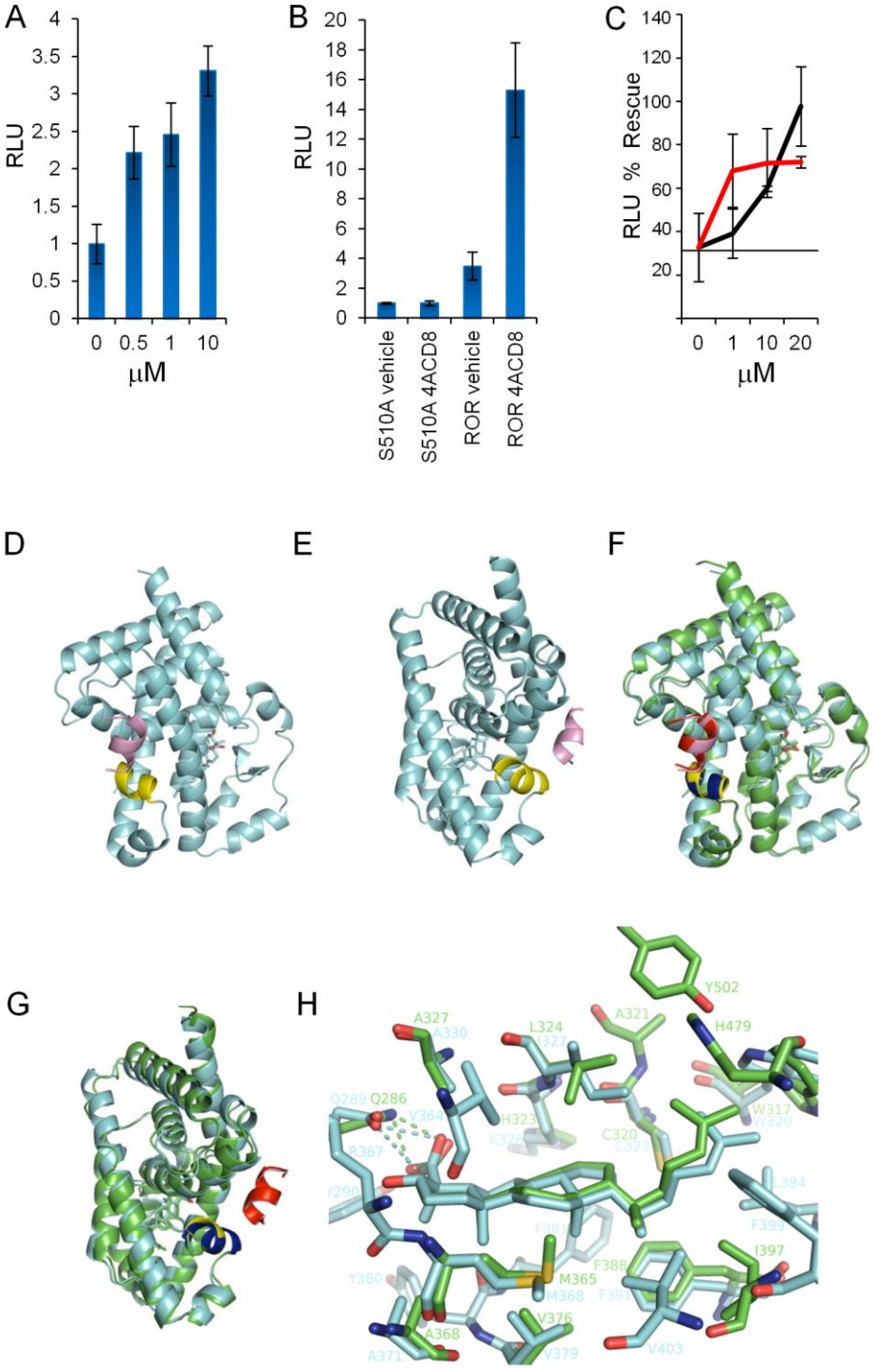
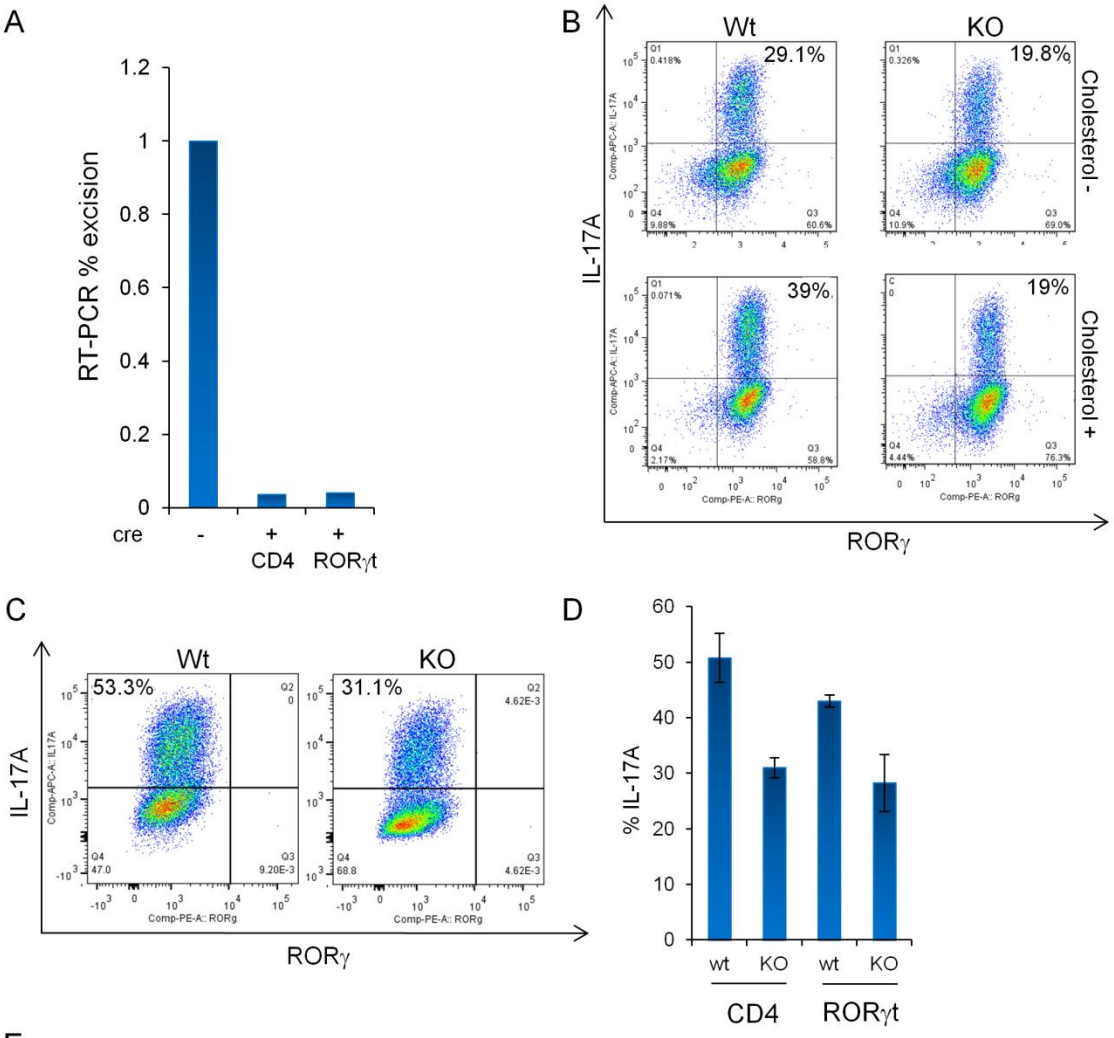


Figure S4 related to figure 4. Carboxysterols are both RORα and RORγ ligands. (A) 4ACD8 induces RORγ specific reporter activity in *Cyp51*$^{-/-}$ fibroblast 1F7. 1F7 cells were transfected with RORγgal4 reporter and after 24 hrs cells were treated overnight with different μM concentrations of 4ACD8 and luciferase reporter activity measured. Vehicle is set at 1. N=2 (B). RORγ reporter activity induced by 4ACD8 is specific. 1F7 cells were transfected as described above with either inactive RORγ mutant S510A or wt RORγ–gal4 fusion proteins. After 24 hrs cells were incubated overnight with 5 μM 4ACD8 and luciferase reporter activity measured. (C) 4ACD8 and 25-hydroxycholesterol are competitors for the RORγ inhibitor ML209 (Huh and Littman, 2012). HepG2 cells were transfected were transfected with RORγ-gal4 reporter . Inhibitor ML209 was added 1-2 hrs prior to competitor 4ACD8 (Black) or 25-hydroxycholesterol (Red). The line indicates the maximum inhibition of ML209 (1 μM). A value of 100% is given to cells without inhibitor. Luciferase activity was read 16 h later. Representative experiment of n=3. (D) Ribbon drawing showing the structure of RORα bound to carboxysterol (4ACD8). The RORα LBD is depicted in cyan with the ligand inside containing oxygen atoms (Red). The RIP-140 coactivator peptide is shown in pink and Helix 12 in yellow. (E) A 180 degree rotation of (D). (F) The RORα and RORγ structures are similar and overlap each other. The RORα LBD is depicted in cyan with the ligand inside containing oxygen atoms (Red). The RIP-140 coactivator peptide is shown in pink and Helix 12 in yellow. The RORγ LBD is depicted in green with the ligand inside containing oxygen atoms (Red). The RIP-140 coactivator peptide is shown in red and Helix 12 in blue. (G) a 180 degree rotation of (F). (H) Detailed view of the conserved interaction of 4ACD8 within the RORγ and RORα ligand-binding pocket. The carboxy group of 4ACD8 forms conserved hydrogen bonds with Q286 (RORγ) and Q289 (RORα) (dashed lines). Atoms Carbon=green (RORγ), cyan (RORα); Nitrogen=blue, Sulphur=yellow and Oxygen=red. The graphs are averages of technical triplicates and the error bars are standard deviations.

Figure S5 related to figure 5



E

CBIs Rescue Th17 polarization in Sc4mol$^{-/-}$ T cells

Compound	Rescue	Experiments
Lanosterol	NR	1
FF-MAS	Yes	1
T-MAS	weak	1
4ACD8	Yes	3
3KZ	NR	3
Zymosterol	NR	3

Figure S5 related to figure 5. Ablation of *Sc4mol* results in defective differentiation of the Th17 cell Lineage. (A) Excision efficiency of *Sc4mol*$^{f/-}$ allele by the CD4-cre and RORγt-cre transgenes. The genomic excision of the floxed allele was determined by RT-PCR of genomic DNA using primers of the unfloxed *Cyp51a1* genomic allele as non-deletion standards. (B) RORγt-cre *Sc4mol*-deficient naïve T cells differ in their ability to differentiate into Th17 cells in CDM with or without cholesterol for 72 h. Representative experiment of n=3. (C) Impaired Th17 cell differentiation in CD4-cre *Sc4mol*-deficient naïve CD4$^+$ T cells. FACS analysis of Th17 cells stained for IL-17A and RORγt 72 h after polarization. Each experiment compares naïve CD4 T cells sorted from *Sc4mol*$^{+/+}$ and *Sc4mol*$^{-/-}$ animals and polarized side by side as technical triplicates. Th17 polarization, representative experiment (n=3). (D) Bar graph comparing % of IL-17A$^+$ T cells in *Sc4mol*$^{+/+}$ and *Sc4mol*$^{-/-}$ under a CD4-cre or RORγt-cre background. Data are quantified as bar graphs of averages of per cent differentiated cells. Representative experiment (n=3). (E) CBIs can rescue Th17 cell differentiation in *Sc4mol*-deficient naïve CD4$^+$ T cells. Rescue was defined as the ability of a compound to increase the number of IL-17A$^+$ T cells to levels comparable to those of wild type. NR=no rescue. Error bars are standard deviations.

Tables S1-3 related to mapping of RORγ ligand biosynthetic pathway in main-text.

Table S1. Metabolic pathways excluded as a source of RORγ activity in insect cell culture in CDM. Metabolic pathways for *Homo sapiens* and *Drosophila melanogaster* in the KEGG metabolic pathway database were compared. Shown are pathways essential for cell growth in CDM or for which insect cells are auxotrophic. **Table S2**. Natural metabolites of mammalian cells tested for RORγ reporter activity in insect and

mammalian based reporter systems. **Table S3**. Genes of Metabolic enzymes, coactivators and corepressors tested for induction or repression of RORγ activity in HEK293T cells by overexpression or shRNA knockdown.

Supplemental Experimental Procedures

Mammalian cell culture: U937 (ATCC CRL-1593.2) cells were grown in RPMI-1640 (Hyclone SH30096.01) and HeLa, HepG2 and HEK293T cells were grown in DMEM (Gibco 11965) supplemented with 2 mM glutamine, 10000 units Pen/strep and 10 % FCS. Cells were tested for mycoplasma contamination with MycoAlert mycoplasma detection kit (Lonza LT07-218). Cholesterol dependency of U937 cells stably transfected with RORγ reporter system (U937ROR) as described below, was checked by comparing the growth and survival of U937ROR cells maintained in serum-free media (1 % BSA, 2 mM glutamine, 2000 units Pen/strep in RPMI-1640 media) in the presence or absence of 1:500 dilution of water soluble cholesterol solution (Sigma S5442). Expression of HSD17B7 in U937 cells was checked by RT-PCR. Cholesterol dependency of fibroblast cell lines was determined by observing survival in media with 5 % delipidated serum.

Th17 cell polarization: *Sc4mol*$^{fl/fl}$ and *Sc4mol*$^{fl/Ko}$ mice were obtained from EUCOMM and breed in C57Bl\6 strain as described to obtain the viable flox/flox alleles (Skarnes et al., 2011) in conjunction with CD4-cre (Lee et al., 2001) or RORγt-cre (Eberl and Littman, 2004). Th17 polarizations were performed as described (Ciofani et al., 2012). Briefly, naïve CD4 T cells CD4+CD25-CD44-CD62L^{+} were sorted from mouse spleen and lymph nodes. Animals (3-8 weeks old females) were sacrificed and spleen and lymph nodes removed. The organs were homogenized and filtered through a 40 μm mesh to remove large debris. Red cells were removed by treatment with ACK lysis buffer (Lonza 10-548E) and remaining cells were blocked with FC block (eBioscience 14-0161-86) for 15 min on ice, washed and stained with anti-mouse CD4 e450 (eBioscience 48-0042-82), CD25 PE-Cy7 (eBioscience 25-0251-82), CD44-PE (eBioscience 12-0441-83) and CD62L-APC (eBioscience 17-0621-83), for 15 min on ice. After incubation cells where washed in MACS buffer (PBS pH 7.4, 0.5 mM EDTA and 1 % BSA), resuspended in MACS buffer containing 1 μg/ml DAPI and filtered through a 40 μm mesh. Live doublet negative CD4+CD25-CD44-CD62L^{+} cells were sorted using the FACS Aria (BD)

equipment. Post-sort purity was checked and cells were spun down and 1 x 10^5 cells/400 μl were resuspended in IMDM (Sigma 13390) media containing 10 % FCS and supplemented with 2 mM glutamine, 10000 units/ml Penicillin/streptomycin, 100 μg/ml gentamicin, 1 x 10^{-5} M β2-mercaptoethanol and antibodies against mouse IL4 (clone (11B11)/IFN-γ (clone XMG1.2) (each 20 μg/ml), CD28 (clone 37.51) (10 μg/ml) and CD3e (clone 145-2C11) (2.5 μg/ml). The equivalent of 1 x 10^5 cells were placed on 48 well plates pre-coated with 1:18 dilution of rabbit anti-hamster IgG antibody (MP Biomedicals 55398) reconstituted in 3 ml of PBS. Plated cells were incubated overnight at 37 ^{0}C 5 % CO_2. Next day 1 ng/ml Tgfβ and 5-20 ng/ml IL-6 was added to media or 90 % of media was replaced with 350 μl of synthetic media prepared with IMDM supplemented with 1x glutamax, 1x Insulin-transferin-selenium-X (Gibco 51500), 20-50 units/ml Penicillin/streptomycin, 10 μg/ml gentamycin, 1 % Pluronic F-68 (Sigma P5556), antibodies against mouse IL4/IFN-γ (each 20 μg/ml), CD28 (10 μg/ml) and CD3e (2.5 μg/ml) 1 ng/ml Tgfβ, 5-10 ng/ml IL-6 with or without synthetic cholesterol solution (Sigma S5442) (1:1000) dilution. After 48 hrs, 400 μl of additional media was added to wells to avoid culture crashing (Total 800 μl). Next morning wells containing synthetic media had media replaced by IMDM with 10 % FCS and we added 40 μl of a 20x mix (PMA (125 μM), Ionomycin (7.2 μg/ml), 1:50 of Golgi STOP solution (BD 554724)) to the wells. Remaining wells could be split at a 1:8 dilution into new anti-hamster IgG pre-coated wells containing complete (antibody plus cytokines) FCS or synthetic media prepared as described above. Cells were stained with acqua for 15 min in PBS on ice, fixed for 30 min on e-bioscience fixation/permeabilization concentrate (eBioscience 00-5123-43) prepared 1:4 in fixation/permeabilization diluents (eBioscience 00-5223-56) washed twice in Perm/wash (BD 51-2091KZ) and incubated with anti-mouse IL17A Alexa 647/APC (eBioscience 51-7177-82/Biolegend 506916) and IFNγ (eBioscience 12-7311-82), FOXP3 (eBioscience 12-5773-82) or RORγ-PE (eBioscience 12-6981-82) following manufacturer instructions.

Rescue experiments were performed by providing the CBIs either as conjugates with recombinant human ApoE3 (Sigma SRP4696) prepared as described in (van den Elzen et al., 2005) or by adding the CBI in lipid mix *TABLE 3 US patent 8198084) at

concentrations of 250-500 μM. The lipid mix was added either to synthetic or FCS containing media during the Th17 cell polarization procedure and analyzed by FACS as described above.

*TABLE 3 (US patent 8198084) Lipid supplement (final concentrations in 1X Replacement medium). CONCENTRATIONS RANGE (G/L) INGREDIENT (G/L) ABOUT (G/L) Pluronic F68 0.1-4.0 1.7 1.74 ethanol 0.09-2.0 0.9 0.87 mL cholesterol 0.0007-0.07 0.007 0.00696 lipoic acid 0.00003-0.003 0.0003 0.000296 linoleic acid 0.00001-0.001 0.0001 0.000122 .alpha.-tocopherol 0.00002-0.002 0.0002 0.000231 palmitic acid 0.0002-0.02 0.002 0.00174 oleic acid 0.0002-0.02 0.002 0.00174 dilinoleoyl 0.0002-0.02 0.002 0.00174 phosphatidylcholine stearic acid 0.0002-0.02 0.002 0.00174 linolenic acid 0.0002-0.02 0.002 0.00174 palmitoleic acid 0.0002-0.02 0.002 0.00174 myristic acid 0.0002-0.02 0.002 0.00174.

Insect cell culture: S2 cells were grown and maintained in Express Five SFM media (Gibco 10486025). Kc 168 were grown and maintained in CDM or SSM1 media as specified. Insect CD formulation was prepared as follows, 40 % of unsupplemented Grace's Insect Medium (Gibco 11595) were supplemented with 10 % Murashige and Skoog basal salt micronutrient solution 10x (Sigma M0529), 1x NEAS (non-essential amino acid solution) 100x (Gibco 11140), 1x Sodium Pyruvate 100 mM (100x) (Gibco 11360), 1x Insulin-Transferin-Selenium A (Gibco 51300-044) and 1-0.5 % (final) Pluronic F-68 (Sigma P5556). CD media can be supplemented with 1x Yeastolate Ultrafiltrate 50x (Gibco 18200) to produce a cholesterol-free semi-synthetic media (SSM1).

Mammalian reporter systems: The Renilla luciferase (RL) plasmid pRL-CMV (promega E2261) was used for normalization. PGL4.31[luc2p/GAL4UAS/Hygro] (luc2p) (Promega C935A) containing Firefly luciferase under GAL4UAS transcriptional control was used in experiments with GAL4 DBD-NHR LBD fusion systems. For full length RORγ reporters the GAL4 UAS sites of PGL4.31 were replaced by 4 RORγ specific RORE elements (Medvedev et al., 1996). For endogenous promoters we used the IL23 receptor enhancer sequence (IL-23res) containing 1 RORγ consensus element (mouse chr6: 67432921-67433595) (Ciofani et al., 2012) cloned into the pGL4min plasmid. Inducible full length RORγ reporter system was produced by inserting RORγ-Ires-eGFP

into the pTRE-tight vector (Clontech PT3720-5). Constructs were inserted into different vector systems as needed: SV40 promoter based pSG424 plasmid(Sadowski and Ptashne, 1989). CMV promoter based pcDNA3.1 vectors (Invitrogen V790-20) and lentiviral vector pTRE-ires-eGFP for tTa regulated promoters (Ivanova et al., 2006). Luciferase activity was measured using the dual luciferase reporter kit (Promega E1960).

Transient transfections: Mammalian cells were plated at a concentration of 5 x 10^4 cells/well in flat bottom 96 well plates. Before transfection, media was replaced to antibiotic-free, 1 % FCS DMEM. Cells were transfected with DNA:Reagent mix prepared in OptiMEM media (Gibco 31985062) using lipofectamine 2000 (Gibco 11668019) for HEK293T and Hela cells, Fugene HD (Roche 04709705001) or 30-80 μl/ml of 1 mg/ml of polyethyleneimine (PEI) (Polyscience 24765) solution for HepG2 cells. PEI was used for all large scale transfections in mammalian cell lines. Reagents were used following manufacturer instructions. Normalization was performed with 1-50 ng/ml of pRL-CMV plasmid, as a firefly reporter we used 0.25-1 μg/ml of luc2p, luc2p_rore or IL-23res plasmids as indicated. The proportion of NHR/RORγ driver plasmid was 2-5x the luciferase reporter vector concentration. For full length RORγ reporter system 0.5-1 μg/ml of tet-off plasmid (Clontech PT3073-5) (tTA-off) was added to the pRL-CMV, luciferase reporter, ROR mix respectively. HEK293T cells were preferred for overexpression experiments and Hela cells had best results for shRNA knockdown experiments. Transfection of transformed fibroblast cells lines 1F7 and sxlt was performed using PEI. For rescue of agonist activity with CBIs we used a combination of 2 μg luc2p luciferase reporter and 4 μg of RORγ-gal4 under a CMV promoter.

Transient transfections in insect cells were performed as follows: S2 or Kc167 cells were plated at 1.5 x 10^5/2.5 x 10^5 cells/well in flat bottom 96 well plates and a DNA mix of 75 μl/well was prepared in CD or SSM1 medium containing (S2-Kc167) 2-5 ng RL under a PolIII promoter for normalization, 5-25 ng of HM1 plasmid were the adenovirus major late promoter of Luc2p was replaced by a minimal *Drosophila melanogaster* (*D.m.*) hsp70 minimal promoter(Pelham, 1982) constructed by annealing of primers hsp70_forw ctagcgagcgccggagtataaatagaggcgcttcgtcgacggagcgtcaatta and hsp70_rev gatctaattgacgctccgtcgacgaagcgcctctatttatactccggcgctcg. The same constructs used for

mammalian transfections were cloned in an insect vector under the *D.m.* actin promoter(Huh et al., 2011) and used at concentrations of 10-50 ng/well. The final transfection mix was prepared by adding 0.5 μl/well of Cellfectin reagent (Invitrogen 10362-010).

Stable RORγ reporter cell line: The cell line U937 (ATCC CRL-1593.2) was transfected by electroporation, with plasmids pRL-CMV, Luc2p and tTA-off and selected with 300 μg/ml Hygromycin and 200 μg/ml neomycin. Clones were selected for expression of Renilla and low levels of firefly luciferase and then infected with lentivirus containing a TRE RORγ-gal4 fusion protein linked to an IRES-eGFP sequence. GFP positive cells were sorted and cloned. Clones were selected for stable expression of genes and regulation of firefly luciferase activity by doxycyclin.

Overexpression screening: The mouse genomics informatics database from Jackson Laboratories (MGI) was used to identify genes involved in the metabolism of lipids in the mouse genome. A list of enzymes was created and then checked for presence in the Open Biosystems IRAV mouse FL cDNA 060105 library. Clones used in this study (Table S3) were confirmed by sequencing. Transfections for the screens were performed in HEK293T cells in 96 well plates with a DNA mix prepared as follows: pRL-CMV 2-10 ng/ml, Luc2p 0.5 μg/ml, RORγ-Gal4 DBD fusion under a SV40 promoter 1 μg/ml and 1 μg/ml of either empty vector or enzyme containing vector. Transfections were performed using Lipofectamine 2000 (Invitrogen 11668-019) following the manufacturer instructions.

Inhibition of Cholesterol Biosynthetic pathways: Inhibition and knockouts in the cholesterol biosynthetic pathway was performed by shRNA knockdown or using CRISPR technology as descrcribed. We used shRNA clones from the TRC1 Mission Sigma library to knockdown *SC4MOL*, *NSDHL*, *NRIP1* and *LCOR*. We also designed additional shRNAs against the human or mouse biosynthetic enzymes using optimized Dharmacon algorithm. Synthetic oligonucleotides were produced and cloned into lentiviral vectors using a strategy previously described (Ivanova et al., 2006). Transfections for HEK293T cells were performed as described in overexpression screening. The cells transfected with shRNA were selected for 5-7 days with 300 μg/ml hygromycin, prior to transfection with RORγ reporter system. The efficiency of the knockdown was determined by RT-PCR and

Western Blot for human CYP51A1. For rescue experiments in human cells shRNA vector was co-transfected with the mouse *Cyp51* cDNA.

Chemicals: Chemicals were purchased from Sigma, Steraloids, Cayman Chemicals, research plus and Avanti polar lipids or directly isolated/synthesized (see below) and tested at various concentrations. Commercially non-available sterols were taken from the Nes sterol collection (Nes et al., 2009). Insect cell ecdysone biosynthetic intermediates were provided by Dr. Rene Lafont (Blais et al., 1996). For rescue experiments squalene was prepared as emulsion in synthetic media (DMEM, ITS-X and Pluronic 1 %) or media containing 2.5-5 % Charcoal/Dextran treated FCS (Hyclone SH30068.02). The emulsion was prepared by diluting squalene (Sigma S3625) in FFAmix (Table 3 US patent 8198084) described in T cell rescue experiments or ethanol and then adding the media with 1% FCS, followed by vortex and 15 minutes incubation in batch sonication. Competition assays with RORγ inhibitors digoxin (Huh et al., 2011) and ML209(Huh and Littman, 2012) were performed with mammalian cells transiently transfected with RORγ-gal4 driving a Gal4 UAS firefly reporter. The cells were incubated 1 hour with inhibitors for RORγ prior to compound addition to culture. Luciferase activity was read out 16 hrs after addition of competitor.

Squalene uptake and metabolization by fibroblasts: NIH 3T3 cell line was obtained from the American Type Culture Collection (ATCC CRL-1658^{TM}) and cultured according to ATCC recommendations. A 6 well plate received 1 x 10^5 cells (40-50% confluence) in DMEM media supplemented with 10% fetal calf serum (FCS) at 37 °C in a humidified 5% (v/v) CO_2 atmosphere for 24 h. The next day the medium was replaced with medium containing 10%, 1% or 0% FCS as indicated. A concentration of 0.5 or 1 µCi (≈ 0.017 – 0.034 µM) of ^{3}H-squalene (Biotrend Chemikalien, ART-0355-10) dissolved in either 0.5% ethanol or 0.01% Tween 80 (Sigma P4780) with vigorous vortexing and 15 min sonication was added to cells for 24 h. The medium was collected, cells washed twice with PBS, scrapped of the well in distilled water and lysed with freeze-thaw cycles. Sterols were extracted two-times with 3 ml ethyl acetate (Sigma 270989), vacuum-dried and dissolved in 250 µL of acetonitrile (J.T. Baker 9012-03).

Samples were analyzed by a reverse phase HPLC (Knauer, Berlin, Germany) and detected with radioactivity detector (Raytest, Ramona 2000). 100 µl sample was injected

onto a LiChrospher column (250 × 4.6 mm) at 25 °C and acetonitrile:water (93:7 (v/v)) at a flow rate of 1 ml/min was used as a mobile phase. Data was analyzed with the Eurochrom HPLC software, version 1.65.

Chemical Synthesis: 4α-carboxycholest-7-en-3β-ol was synthesized as described previously (Rondet et al., 1999). 4-methylcholesta-4,7-dien-3-one and 4-methylcholest-4-en-3-one were synthesized as described in the literature (Crabtree et al., 1990; Mazur and Sondheimer, 1958; Rondet et al., 1999). These were carboxylated by established protocol to 4α-carboxy-4β-methylcholest-7-en-3β-ol and 4α-carboxy-4β-methylcholestan-3β-ol in synthetic schemes outlined below. 4α-methylcholesta-8,24-dien-3-one was quantitatively produced by Jones oxidation of 4α-methylcholesta-8,24-dien-3β-ol with established protocol (Nes et al., 2009).

Sterols were quantified by previous methods and purity was tested by gas chromatography-mass spectrometry (30 m HP-5 capillary column coupled to HP 6890 gas chromatograph interfaced to a 5973 mass spectrometer at 70 eV; GC flow rate of He was set at 1.2 ml/min, injector port was 250 ^{o}C and the initial temperature was set at 170 ^{o}C, held for 1 min, and increased at 20 ^{o}C/min to 280 ^{o}C) as previously described(Miller et al., 2012). Chromatographic data is presented relative to cholesterol (RRT*c*) and is given for carboxylates as the methyl esters. Substrates were of at least 95 % purity by ion count. Purification of compounds was conducted by gravity-flow chromatography (silica gel, standard grade 60-230 μm particle size, BDH Chemicals, London) or semipreparatory TLC (silica gel, 1000 μm, Analtech, Wilmington, DE) in 85:15 hexanes/ethyl acetate, single development. Products were visualized with 2 N aqueous sulfuric acid and extracted with 1:1 (v/v) ethyl acetate/diethyl ether. Chromatographic data for carboxylates are reported as the methyl esters.

Preparation of 4α-carboxy-4β-methylcholest-7-en-3β-ol. Briefly, 4α-carboxy-4β-methylcholest-7-en-3-one prepared with 44.0 mg 4-methylcholesta-4,7-dien-3-one was dissolved in ether (distilled from sodium and benzophenone ketyl) with *tert*-butanol and added to lithium amide-dried ammonia at -78 ^{o}C, carboxylated with methyl cyanoformate in dried diethyl ether at -78 ^{o}C to yield 4α-carboxy-4β-methylcholest-7-en-3-one methyl ester (19.2 mg, 38 %). RRT*c* 1.82. MS *m*/*z* (rel. int.) M^+ = 456 (7), 438 $[M\text{-}H_2O]^+$ (12), 423 $[M\text{-}Pr]^+$ (12), 397 $[M\text{-}CO_2\text{-}Me]^+$ (7), 378 $[M\text{ -}H_2O\text{-}CHOOMe]^+$ (6),

363 [M-CHOOMe-Pr]$^+$ (4), 313 [M-A ring-H]$^+$ (64), 144 (100). The final synthesis of 4α-carboxy-4β-methylcholest-7-en-3β-ol used 9.2 mg 4α-carboxy-4β-methylcholest-7-en-3-one that was reduced by sodium borohydride to yield 9.6 mg epimeric 4α-carboxy-4β-methylcholest-7-en-3-ol in 1:3 α-β ratio as the methyl esters (7.1 mg, 50 %). Epimers and ketone were separated by TLC. 4α-carboxy-4β-methylcholest-7-en-3β-ol free acid was produced by saponification of the methyl ester by 10:10:80 potassium hydroxide–water-methanol (w/v/v). RRT*c* 1.99. EIMS *m/z* (rel. int.) M^+ = 458 (12), 440 [M-H_2O]$^+$ (47), 425 [M-Pr]$^+$ (19), 398 [M-CHOOMe]$^+$ (51), 380 [M -H_2O-CHOOMe]$^+$ (13), 365 [M-CHOOMe-Pr]$^+$ (100), 314 [M-A ring]$^+$ (30).

Synthesis of 4α-carboxy-4β-methylcholestan-3β-ol from 4α-carboxy-4β-methylcholestan-3-one: 40.0 mg 4-methylcholest-4-en-3-one was carboxylated with methyl cyanoformate in diethyl ether (distilled from sodium and benzophenone ketyl) at 78 ^{0}C to yield 4α-carboxy-4β-methylcholestan-3-one methyl ester (18.4 mg, 40 %). RRT*c* 1.71. EIMS *m/z* (rel. int.) M^+ = 458 (27), 440 [M-H_2O]$^+$ (17), 430 [M-CO]$^+$ (3), 399 [M-CO_2-Me]$^+$ (13), 383 [M-CHOOMe-Me]$^+$ (13), 371 [M-CO_2-CO-Me]$^+$ (7), 315 [M-A ring-H]$^+$ (24), 143 [A ring+H]$^+$ (100). The final synthesis of 4α-carboxy-4β-methylcholestan-3β-ol by reducing 18.4 mg 4α-carboxy-4β-methylcholestan-3-one with sodium borohydride to yield 10.2 mg epimeric 4α-carboxy-4β-methylcholestan-3-ol in 1:3 α-β ratio as the methyl esters (41 %). Epimers and ketone were separated by semipreparatory TLC. 4α-carboxy-4β-methylcholestan-3β-ol free acid was produced by saponification of the methyl ester by 10:10:80 potassium hydroxide-water-methanol (w/v/v). RRT*c* 1.83. EIMS *m/z* (rel. int.) M^+ = 460 (21), 442 [M-H_2O]$^+$ (13), 428 [M-MeOH]$^+$ (28), 417 [M-Pr]$^+$ (13), 410 [M -H_2O-MeOH]$^+$ (8), 403 [M-Bu]$^+$ (100), 400 [M-CHOOMe]$^+$ (9), 385 [M-CHOOMe-Me]$^+$ (8), 383 [M-H_2O-CO_2-Me]$^+$ (11), 315 [M-Aring-H]$^+$ (25).

Synthesis of 4β,14α-dimethylcholesta-7,24-dien-3,30-diol: was synthesized starting from lanosterol by the reported method for the site selective γ-functionalization of primary C-H bonds controlled by a hydroxyl group (Simmons and Hartwig, 2012).The acidic workup upon completion of the synthesis resulted in the isomerization of the Δ^8 to Δ^7 isomer.

Synthesis of 4α-formyl-4β,14α-dimethylcholesta-7,24-dien-3-ol: This compound was isolated from the crude reaction mixture of compound 4β,14α-dimethylcholesta-7,24-dien-3,30-diol. Both compounds were purified initially by semi-preparatory HPLC on an ISCO HPLC system (Model 2350) equipped with a Phenomenex C18-Selectosil column (250 mm x 10 mm x 10 μm) (Phenomenex, Torrance CA) and operated with 100 % methanol with a flow rate of 1 mL/min at 25 ^{o}C and elutions were monitored by ultraviolet (UV) absorbance at 210 nM. Further purification was carried out by analytical HPLC using an Agilent 1100 HPLC system (Agilent, Santa Clara CA) equipped with a Phenomenex C18-Luna column (250 mm x 4.6 mm x 5 μm) operated with 100 % methanol at 30 ^{o}C and a flow rate of 1 mL/min. Elutions were monitored by UV absorbance at 210, 242 and 282 nm. The mass spectra presented herein were subject to background subtraction. RRT*c* values of synthesized and calculated standards: Δ8 RRT*c* = 1.73 (OH) and 1.84 (aldehyde), Δ7 RRT*c* = 1.83 (OH) and 1.95 (aldehyde).The Δ8 RRT*c* values were determined by dividing the synthesized Δ7 RRT*c* values by 1.06. The GC-MS spectra of 4β,14α-dimethylcholesta-7,24-dien-3,30-diol and 4α-formyl-4β,14α-dimethylcholesta-7,24-dien-3-ol are shown in (supplemental experimental procedures figure 1).

The compound 4α-carboxy-4β-methyl-cholest-8,24-dien-3β-ol was isolated from the *Erg26* yeast strain (Mat a *ade5, his3, leu2-3, ura3-52, erg26*Δ*::TRP1 trp1::hisG* Δ*hem1*) (Gachotte et al., 1998) as previously described (Rahier et al., 2006) and had the expected spectra for MS : *m/z* (rel.int.) M+= 456(100), 441(47), 438(21), 423(27), 396(23), 363(23), 285(19), 225(20) and 400MHz 1H NMR of **[1]**, (CDCl3) ; δ: (ppm) relative to tetramethylsilane ; J(Herz):0.588(3H,s,H18),0.939(3H,d,J=6.4,H21),1.008(3H,s,H19),1.176(3H,s,H4β),1.600(3H,s,H26),1.680(3H,s,H27),4.021(1H,dd,J=16,J=7.5,H3α)5.090(1H,tt,J=7.1,J=1.3,H24).

Quantitation of C4-modified canonical CBIs in bovine thymus: Carboxysterol analysis of lyophilized thymus: The sample of lyophilized calf thymus (20 grams dry weight) was extracted with dichloromethane/methanol 2:1 for three hours at 65 °C, evaporated to dryness and saponified with 6 % KOH in methanol for 2 hours at 65 °C. The mixture was then diluted with water and extracted with ethyl-acetate, first at pH > 12

and then at pH < 2, to be sure to extract quantitatively the carboxysterols. The extract was evaporated to dryness and thin layer chromatographied on SiO_2 plates using dichloromethane. Only two fractions were visible on the plates and collected: Fraction 1 and fraction 2. Fraction 1 was eluted and treated with diazomethane and then acetylated to produce 4-carbomethoxysteryl 3-acetate and 4-acetoxymethylsteryl 3-acetate. Fraction 2 was eluted and acetylated. The derivatized fraction 1 was separated by thin layer chromatography on SiO_2 plates using dichloromethane as a solvent, leading to 3 visible bands : band 1 (Rf = 0-0.09), band 2 (Rf= 0.09-0.23), and band 3 (Rf= 0.23-0.35). Fraction 2 and bands 1, 2, and 3 were analyzed by GC-MS with an electron energy of 70 eV and 15 eV. Fraction 2 contained cholesterol and band 3 contained sterol lipids. The relative retention time relative to cholesterol (RRTc =1) on a DB-5 column for lanostenyl-acetate (M+ = 470) is 1.26 and the RRTc of 4-carbomethoxy-4-methyl-cholest-8,24-diene-3-beta-yl-acetate (M+ = 498), authentic standard, is 1.49. The 4α-methyl-hydroxylzymosterol derivative was unambiguously identified as 4-acetoxymethyl-cholest-7-ene-3-beta-yl-acetate : RRTc = 1.51 ; M+ = 500(3), 485(1), 440(80), 425(7), 380(100), 365(53), 327(6), 312(14), 267(27). The MS of this compound is identical with that of an authentic synthetic standard of 4-acetoxymethyl-cholest-7-ene-3-beta-yl-acetate analyzed in parallel. The concentration of this compound in the thymus was estimated at 50 nM. Other potential carboxysterols could not be unambiguously identified by this technique.

For 4ACD8 estimates the 4 grams of thymus where lyophilized. This produced 700 mg of dry material which was resuspended in 1.5 ml methanol, 1 ml of 60% KOH and 1ml of 0.5% pyrogallol for every 300 mg of dry weight tissue. Coprostanol (10 μM) was added as an extraction control. The material was heated for 2 h at 85 ^{0}C. The nonsaponified material was extracted 2x with petroleum ether:hexane (1:1). The organic phase was collected and dried under N2, resuspended in 50 μl ethanol and diluted 1:10 or 1:100 in dichlorometane, analyzed by GC-MS and the results scanned by single ion monitoring (SIM) and NIST library scan. The final estimated concentration was calculated by defining (concentration standard/peak area standard) x peak area sample. This value was multiplied by dilution and adjusted for the efficiency in recovery of coprostanol extraction control (7.5%). The calculated concentrations for peaks of *mz* 442

with RT similar to that of the 4ACD8 standard ranged from 0.15 to 0.5 μM in the thymus.

Simulation of expected concentrations of the canonical CBI 4ACD8 in cells: As a first step we define the Steady-State equation for the rate of formation and decomposition of [4α-carboxy-4β-methyl-cholesta-8,24-dien-3beta-ol(4ACD8)]. The general rate equation for 4ACD8 based on the above scheme is:d[4AD8]/dt = "rate of formation" − "rate of decomposition" (1). Modeling the rate of "formation" and "decomposition" as a Michaelis-Menton-type process we have: d[4ACD8]/dt= k_{cat}^{sc4mol} [$SC4MOL$]$_t$[4ACD8]/[4ACD8] + K_m^{sc4mol} − k_{cat}^{NSDHL} [$NSDHL$]$_t$[4ACD8]/[4ACD8] + K_m^{NSDHL} (2). which at steady state the "rate of formation" can be simplified to the rate determining step (flux) of the pathway as $Flux \approx V_{MAX}^{HMGCR} = k_{cat}^{HMGCR}[HMGCR]_t$ (3) at which the concentration of 4ACD8 doesn't change with time and d[4AD8]/dt= 0 (4). Thus, the steady state assumption simplifies equation 2 – via equations 3 and 4 – to equation 5 as 0 = $k_{cat}^{HMGCR}[HMGCR]_t$ − k_{cat}^{NSDHL} [$NSDHL$]$_t$[4ACD8]/([4ACD8] + K_m^{NSDHL}(5). In addition, for non-rate determining steps the [Substrate] << Km which allows further simplification: 0 = $k_{cat}^{HMGCR}[HMGCR]_t$ −k_{cat}^{NSDHL} [$NSDHL$]$_t$ [4ACD8]/K_m^{NSDHL}(6). Rearranging equation 6 we obtain an expression for the concentration of 4ACD8 at steady state:

[4ACD8] =$k_{cat}^{HMGCR}[HMGCR]_t K_m^{NSDHL}/k_{cat}^{NSDHL}$ [$NSDHL$]$_t$ (7). Equation 7 can be rearranged so that we can estimate the concentration of 4ACD8 by using biochemical constants only into [4ACD8] = K_m^{NSDHL} $k_{cat}^{HMGCR}/k_{cat}^{NSDHL}$X[$HMGCR$]$_t$/[$NSDHL$]$_t$ (8). Absolute expression levels of enzymes are not available but the ratio of expression levels can be estimated based on RNAseq data. For instance in Th17 cells, according to RNAseq data (Ciofani et al., 2012): [HMGCR]/[NSDHL] ≈ 10 therefore equation 8 is simplified into [4ACD8] = 10. K_m^{NSDHL} $k_{cat}^{HMGCR}/k_{cat}^{NSDHL}$ (9). Based on published measurements *in vitro*: $K_m^{NSDHL} \approx 7\mu M$ (10) (Rahimtula and Gaylor, 1972), k_{cat}^{NSDHL} = $0.2s-1$ (11)(Rahimtula and Gaylor, 1972) and k_{cat}^{HMGCR} = $0.05s-1$ (12) (Bhattacharya et al., 2014). If *SC4MOL* and *NSDHL* diffused freely (i.e. *in vitro*) we would estimate [4ACD8] ≈ $14\mu M$ (13). However, because *SC4MOL* and *NSDHL* are tethered near each other by the ER and a scaffold protein we would expect a 100-1000x increase in the NSDHL k_{cat} ((Mo et al., 2002); (Mialoundama et al., 2013)*)* which lowers our estimate at

least 100x to$[4ACD8] \approx 140nM$ (14). This estimate is similar to concentrations of other CBI's which have similar k_{cat}s and K_m's like CYP51 (Quehenberger et al., 2010). Finally, the concentration of [4ACD8] can be estimated using both flux measurements and biochemical parameters so that equation (7) $[4ACD8] = k_{cat}^{HMGCR}[HMGCR]_t K_m^{NSDHL}/k_{cat}^{NSDHL}[NSDHL]_t$ can be set in terms of Vmax's as $[4ACD8] = K_m^{NSDHL} \times k_{cat}^{HMGCR}[HMGCR]_t/k_{cat}^{NSDHL}[NSDHL]_t = K_m^{NSDHL} \times V_{max}^{HMGCR}/V_{max}^{NSDHL}$ (15). Since Vmax's are often not directly measured, it is easier to find "specific activity" measurements $SA_{max}^{HMGCR} \approx V_{max}^{HMGCR} / mg \approx 50pmol$ x min^{-1} x mg^{-1}(Aboushadi et al., 2000; Edwards et al., 1972; Quehenberger et al., 2010; Shapiro and Rodwell, 1971; Tanaka et al., 1982); $SA_{max}^{NSDHL} \approx V_{max}^{NSDHL} / mg \approx 600pmol$ x min^{-1} x mg^{-1} (16) (Rahimtula and Gaylor, 1972). Because both of these numbers are in terms of mg of total protein the mg term cancels out in the V_{max} ratio in equation 15 so that $[4ACD8] = K_m^{NSDHL} \times V_{max}^{HMGCR}/V_{max}^{NSDHL} = 7 \text{ x } 10^{-6}\ \mu M \times 50pmol$ x min^{-1}x $mg^{-1}/600pmol$ x min^{-1}x mg^{-1} (17). This gives us an estimate which is within the same order of magnitude of our first $[4ACD8] \approx 580nM$ (18). That again is similar to concentrations of other CBI's whose decomposing enzymes have similar kcats and Km's like *CYP51A1* (Quehenberger et al., 2010).

Identification of non-canonical CBIs in bovine thymus: Fresh bovine thymus was baked overnight at 124 °C to determine % water of tissue. Weight of tissue was determined gravimetrically and volumes by water displacement in a graduated cylinder. Lyophilized and baked thymus were separately saponified at reflux in 10:10:80 (w/v/v) potassium hydroxide:water:methanol for 90 min. The non-saponifiable fraction (NSF) was then added to an equivalent of water and extracted with two equivalents of mixed hexanes three times (Goad and Akihisa, 1997). Hexanes were removed *in vacuo* by rotary evaporator and transferred with acetone. Sterols were quantified by GC-FID as previously described (Xu et al., 1988).

Total lipids from thymus were fractionated by semi-preparatory HPLC on a DionexUlti-mate 3000 HPLC system (Thermo Fisher, Waltham MA) equipped with a Phenomenex C18-Luna column (250 mm x 10 mm x 10 μm) (Phenomenex, Torrance CA) and operated with 100 % methanol. Analytical HPLC was performed on an Agilent 1100 HPLC system (Agilent, Santa Clara CA) equipped with a Phenomenex C18-Luna

column (250 mm x 4.6 mm x 5 µm) operated with 100 % methanol at 25 ºC and a flow rate of 1 ml/min. Elutions were monitored by ultraviolet (UV) absorbance at 210, 240 and 282 nm. Sterol samples were dissolved in methanol and/or acetone, depending on solubility, and injected at 8.5 µl of unknown concentration for GC-MS. Gas chromatography was performed as described in GC-MS section. The background of the GC-MS was subtracted and common sterol peaks (m/z 69, 83, 95) were removed by the technique. The diagnostic peaks for the 440.36 compound are: 440 $[M]^+$, 425 $[M\text{-}CH_3]^+$, 422 $[M\text{-}H_2O]^+$, 412 $[M\text{-}CO]^+$, 407 $[M\text{-}CH_3\text{-}H_2O]^+$, 379 $[M\text{-}CH_3\text{-}H_2O\text{-}CO]^+$, 377 $[M\text{-}CH_3\text{-}H_2O\text{-}HCO\text{-}H]^+$, 314 [M-side chain (SC) - $CH_3]^+$, 313 $[M\text{-}SC\text{-}H]^+$, 266 $[M\text{-}SC\text{-}CH_3\text{-}H_2O\text{-}HCO\text{-}H]^+$, 259 [M-LC-42 (D cycle fragment of sterols) $–CO]^+$, 257 $[M\text{-}SC\text{-}42\text{-}HCO\text{-}H]^+$.

The concentration of non-canonical CBIs was estimated by using the previously determined RRT*c* values of the oxylanosterol standards as a guide for the GCMS TIC trace of thymus oxysterols that was then searched using selected ion monitoring (SIM) for peaks of *mz* 440 *mz* 442, corresponding to the the 4α-hydroxymethyl or 4α-formyl lanosterol derivatives 4α-formyl-4β,14α-dimethylcholesta-7,24-dien-3-ol, 4β,14α-dimethylcholesta-7,24-dien-3,30-diol, 4α-formyl-4β,14α-dimethylcholesta-8,24-dien-3-ol and 4β,14α-dimethylcholesta-8,24-dien-3,30-diol. Only one unequivocal match was obtained (RRT*c* = 1.84) which corresponded to 4β,14α-dimethylcholesta-7,24-dien-3,30-diol. The lower estimate of this compound in the thymus was calculated to be 60 nM.

Tissue and embryo preparation and cryosections: E14.5 *Cyp51*$^{flox\text{-}FRT\text{-}neo}$ embryos (wild type n=3, *Cyp51*$^{-/-}$ n=4) were obtained, processed and stained as described (Keber et al., 2011; van de Pavert et al., 2009). In short, they were fixed in 4 % formalin at room temperature for 45 min, cryoprotected in sucrose and cryosectioned at 8 µm. Every 10th section was used for screening of LN anlagen, after which three sections located within 120 µm of the area detected in the screening stained using markers for lymph node inducer cells, thus covering the complete lymph node anlagen. During the staining procedure the investigator coded the sections so that the subject determining the phenotype and counting of the cells was "blinded" to the genotype of each section. Cells were counted in the sections containing the largest lymph node anlagen, lymph node inducer cells ($CD45^+Il7r\alpha^+CD4^+$) and precursor lymph node inducer cells

($CD45^+Il7r\alpha^+CD4^-$). For the staining we used mAb clones GK1.5 (anti-CD4), MP33 (anti-CD45) and A7R34 (anti-Il7rα; kindly provided by S. Nishikawa). Mabs were affinity purified from hybridoma cell culture supernatants with protein G-Sepharose (Pharmacia, Uppsala, Sweden) and either labeled with Alexa-Fluor 488, 555 or 647 (Invitrogen, Breda, the Netherlands) or visualized with a secondary antibody conjugated to Alexa-Fluor 488 or Alexa-Fluor 647. Sections were analyzed on a TCS SP2 confocal laser-scanning microscope (Leica Microsystems) and Photoshop (CS3; Adobe). Statistical significance was determined using an unpaired Student's *t*-test.

RORγ Immunoprecipitation (IP) and lipidomics: For endogenous IP, AKR1 thymoma was grown in RPMI-1640 media with 10% FCS. Cells were expanded in 75 and 150 cm^2 flasks (Falcon 353133 and 355001) and then expanded in 850 cm^2 liter Roll over bottles (Falcon 353007). In IPs using recombinant systems HEK293T cells were plated in 10 cm dishes in DMEM with 10 % FCS and transfected with 3xFlag-LBD constructs as described(Chakravarthy et al., 2009). Briefly, transfection was performed with DNA:Reagent mix prepared in serum-free DMEM using 1 μg/ml of 3Flag-RORγ-LBD and empty pcDNA3.1 vector with 80 μl/ml of PEI (1 μg/ml) (Polyscience 24765). Nuclear extract was prepared from HEK293T cells (48 hrs post-transfection) and used at 1 x 10^8 cell equivalents/IP or $3.5x10^{10}$ AKR1 cells/IP at a concentration of $1x10^9$ cells/ml extraction buffer. Briefly, cells were washed in PBS and resuspended in 20 nM Hepes pH 7.9, 1.5 mM MgCl, 10 mM KCl, 0.1 mM PMSF, 1 mM DTT and protease inhibitor cocktail (Roche 11873580001) and incubated on ice for 15-20 min. The proportion of cells per ml buffer was 1 x 10^8cells/ml for HEK293T cells. This proportion was kept constant for the whole procedure. After incubation cells where homogenized 6-7 times with a dounce homogenizer, placed in eppendorf and spun 15000 xg for 15 min. Supernatant was removed and pellet resuspended in 20 mM Hepes pH 7.9, 1.5 mM MgCl, 0.2 mM EDTA, 25 % Glycerol, 420 mM KCl , 0.1 mM PMSF, 1 mM DTT and protease inhibitor cocktail (Roche 11873580001). Pellet was homogenized 7-8 times with a dounce homogenizer, and placed on rotator shaker at 4 ^{0}C for 1-2 hrs. After incubation extract was spun for 30 min at 15000 xg. Supernatant was transferred to a new tube and processed for immunoprecipitation. Digoxin competition experiments were performed by pre incubation of HEK293T RORγ-LBD nuclear lysate for 30-60 min with 30 μM

digoxin. After which digoxin containing samples as well as digoxin negative samples were processed for IP as follows: Nuclear extract of HEK293T cells was immunoprecipitated by adding 40 μl of anti-Flag M2 magnetic beads (Sigma M8823) while nuclear extract of AKR1 cells was immunoprecipitated with 5mg/ml of purified hamster anti-mouse RORγ mab (Sun et al., 2000) coupled to protein-A magnetic beads (Pierce 21348). CTRL were protein-A magnetic beads coupled with Hamster-Ig isotype CTRL. The immunoprecipitation was incubated in rotator shaker for 1 hr to overnight at 4 ^{0}C. After incubation beads were washed 4 times with 50 mM Tris HCl pH 7.4, 100 mM NaCl plus Roche inhibitor cocktail. Elution of flag tagged protein was performed for 30 min with 150 ng/ml of 3xFLAG peptide (Sigma F4799). RORγ isolated from AKR1 cells was eluted with 3M KCl. An aliquot was removed for western blot and the remainder was used for lipid extraction by adding 0.9 % saline and overnight incubation in 2:1 mix of chloroform: methanol (Folch et al., 1957).

For HEK293T cells 3-5 IPs for each set of experimental conditions was processed simultaneously by LC-MS. The whole material of the AKR1 IP was used in a single run. LC-MS was performed on Agilent 6420 Q-TOF coupled to an 1100 liquid chromatography stack. A column =2.1 x 100 mm Agilent Zorbax Eclipse C18 was used with injection volume of 8 μl. Mobile phase: A=H2O/0.1 % formic acid B=ACN/0.1 % formic acid on a gradient T0=95:5, T=15 5:95, T=20 off with a 7 min post run to re-equilibrate. Scan was performed on mass range from 100-1700 *m/z* under source conditions: Drying gas temp=35 ^{0}C, drying gas flow=10 L/min, nebulizer pressure=25psi. Capillary voltage =4000V, fragmentor voltage =150V. Final data from the LC-MS run was analyzed using XCMS package (Smith et al., 2006). The run for endogenous IP (AKR1) was analyzed by running XCMS comparing AKR1RORγ to combined negative controls of AKR1, HEK293T and HEK293T cell lysates treated with digoxin. Peaks common to negative controls were filtered out.

GC-MS: Lipids from RORγ immunoprecipitations (6-15x10^{8}cells) was extracted by adding approximately 1 ml of hexane and sonicating the sample for ~5 min while being gently agitated. Two miniature silica gel columns were prepared using glass Pasteur pipettes filled to approx 2.5 cm of silica per column. Each column was pre-eluted with ~10 ml of hexanes, each sample dissolved in hexanes were added to respective pipets.

Each column with respective sample was washed with 3 ml hexanes and then samples were eluted with absolute ethanol (approximately 6 ml). The ethanol was stripped under nitrogen stream at 45 ^{o}C and then the sample was brought back up in 50 μl of acetone being careful to allow all the solvent to re-suspend any sample that was dried to the sides of the vial. By the time each vial with acetone was agitated as such, only approx 10-12 μl of acetone remained in each vial. 5 μl of the sample were sampled for GC-MS analysis. Gas chromatography was performed on a HP 6890 GC interfaced to an HP5973 electron-impact mass spectrometer (70 eV EI, scan range 50-550 amu) equipped with 0.25 mm i.d. by 30 m fused silica column coated with Zebron ZB-5, film thickness 0.25 μm (Phenomenex, Torrance CA) with He as carrier at a flow rate of 1.2 ml/min. The injection port was 250 ^{o}C, and oven was programmed to be isothermal the first minute, and then ramp to 280 ^{o}C at 20 ^{o}C/min. The quadrupole temperature was 150 ^{o}C; the source temperature was 230 ^{o}C. Retention times are given relative to cholesterol (RRT*c*)[17]. Cholesterol at these conditions elute at 14.1 min.

Expression and Purification of RORα-LBD and RORγ-LBD Proteins: Human RORα-LBD (residues 271–523) franked by an N-terminal 6x His-tag and a thrombin cleavage site was expressed from a pET46 Ek/Lic vector in BL21-CodonPlus(DE3)-RIL *E. coli* cells (Stratagene). The cells were induced with 1 mM IPTG at 20 °C for 20 hrs, collected and lysed in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20 mM imidazole and 10 % glycerol. The supernatant was loaded to HisTrapTM FF crude 5 ml column on AKTAxpress HPLC system. The protein was eluted with a gradient of 20–500 mM imidazole and was dialyzed overnight against 50 mM Tris-HCl (pH 7.5), 100 mM NaCl and 5 mM DTT. Only for crystallization purpose, the 6x His-tag was cleaved by thrombin in the dialysis bag. The protein was further purified by anion exchange chromatography on Q Sepharose^{TM} Fast Flow resin (Amersham Biosciences) and size exclusion chromatography on HiLoad^{TM} 16/60 Superdex^{TM} 200 prep grade column, in which RORα-LBD ran as a monomer. The final protein sample was in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl and 10 mM DTT.

Human RORγ LBD (residues 262–518) with an N-terminal His-tag and a thrombin cleavage site, was expressed in BL21-CodonPlus(DE3)-RIL *E. coli* cells (Stratagene) using a pET46 Ek/Lic vector. The cells were induced with 0.5 mM IPTG at

16 °C for 12 hours, then collected and lysed in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20 mM imidazole and 10 % glycerol. Supernatant was loaded onto a HisTrap™ FF crude 5 ml column on an AKTAxpress HPLC system. The protein was eluted with a 20–500 mM imidazole gradient and dialyzed overnight against 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 10 mM DTT. Only for crystallization purpose, the 6x His-tag was cleaved by thrombin in the dialysis bag. The protein was further purified using a Q Sepharose™ Fast Flow anion exchange column (Amersham Biosciences). The flow-through fractions containing RORγ LBD protein were pooled and concentrated. Subsequent size exclusion chromatography was performed on a HiLoad™ 16/60 Superdex™ 200 prep grade column. The final protein sample was in 20 mM Tris-HCl (pH 8.0), 200 mM NaCl and 10 mM DTT.

Crystallization, data collection and structure determination of ROR-LBDs bound with 4ACD8: The final diluted protein samples of RORα-LBD and RORγ-LBD, after size-exclusion column and with 10 μM 4ACD8 added, were concentrated to 3 mg/ml respectively. 3-fold molar excess of the LXXLL motif peptide derived from RIP-140 residue 498-509 (TLLQLLLGHKNE) (Anaspec) were combined and incubated with both proteins at 4 °C. For crystallization, crystals were grown in hanging drops at 9 °C by mixing 1 μl of the protein-ligand complex with 1 μl of well buffer that contained 200 mM $MgCl_2$ and 9-21 % PEG 3350 for RORα-LBD, and 0.3 M potassium sodium tartrate for RORγ-LBD respectively.

Diffraction data at 100K for both crystals were collected at the Argonne National Laboratory APS beamline SER-CAT 22-ID, and processed with HKL-3000 (Minor et al., 2006). The structures were solved by molecular replacement in the program PHASER (McCoy et al., 2007) using previously determined LBD structures as search models (PDB ID 1N83 for RORα and 3KYT for RORγ respectively). The initial models were rebuilt manually with COOT (Emsley and Cowtan, 2004) and were refined using both PHENIX (Adams et al., 2002) and CNS 1.3(Brunger et al., 1998). The data collection and refinement statistics are listed in Supplemental Experimental Procedures Table I.

Fluorescence polarization-based ligand binding competition assays: 100 nM of final protein samples, RORα-LBD or RORγ-LBD in its corresponding buffer respectively, were incubated with 5 nM fluorescein-labeled 25-hydroxycholesterol and a serial dilution

of each testing compound at 4 °C for 6 hours. The samples were transferred to 96-well black polystyrene plates and the fluorescence polarization signals were measured on FlexStation 3. All the experiments were performed in triplicates and the converted fluorescence anisotropy values were normalized as fractions bound with the IC 50 value of each tested compound determined by curve fitting in GraphPad Prism 5.

Peptide labeling and fluorescence polarization-based peptide recruitment assays: 10 μg of the synthetic peptides (AnaSpec), NCOA2 (sequence EKHKILHRLLQDSY) was mixed with fluorescein-5-EX succinimidyl ester at a molar ratio of 1:2 in 100 mM potassium phosphate (pH 6.8) coupling buffer for 1-hour reaction at 37 °C, quenched by adding 100 mM Tris (pH 8.2) and additional 30 min incubation at room temperature. The labeled peptides were purified using Sephadex^{TM} G-15 columns.

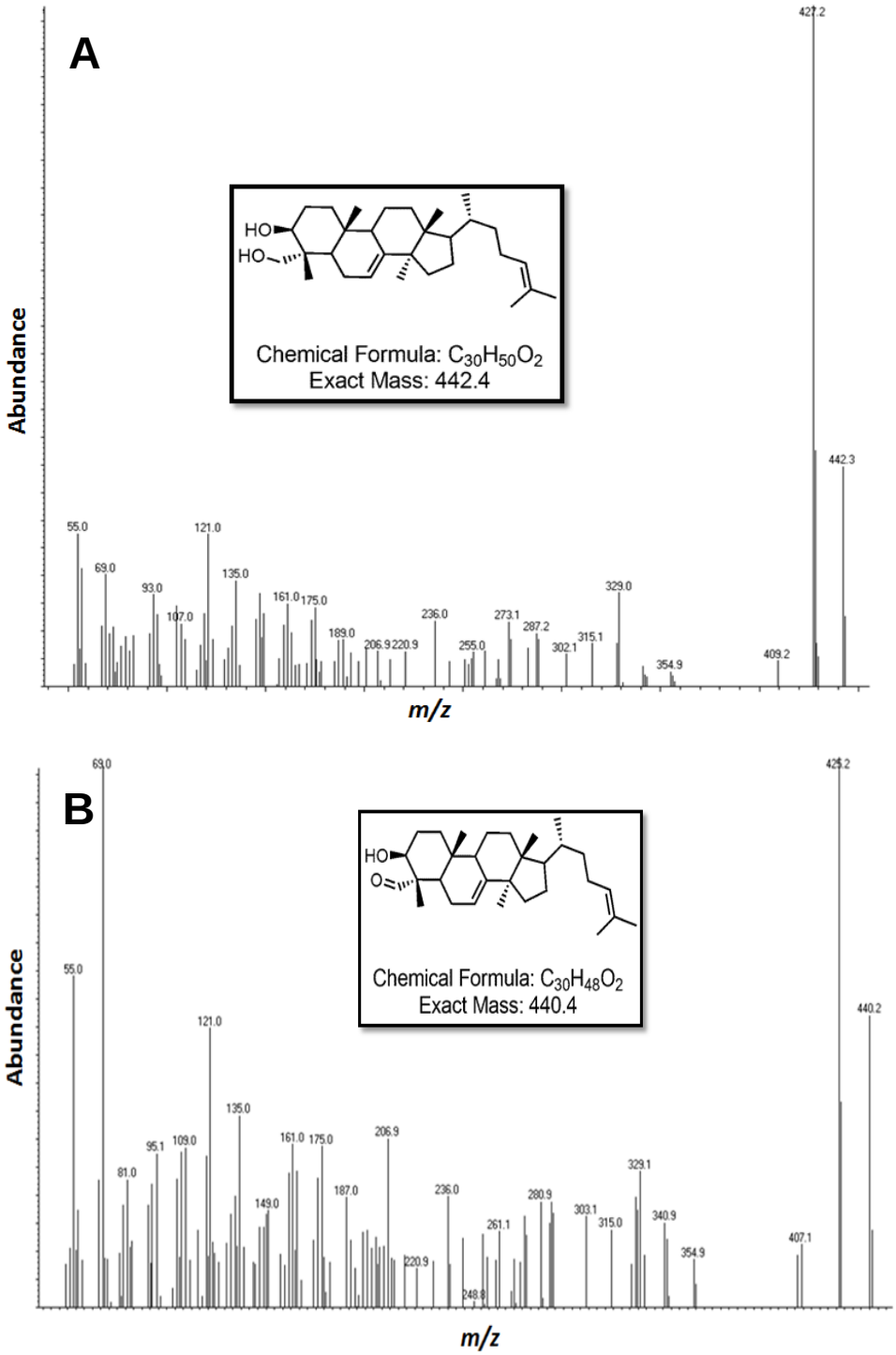
For binding assays, 5 nM of fluorescein-labeled peptide, NCOA2 was incubated with purified RORγ-LBD protein samples in its apo-form or saturated by each individual compound tested for an hour at room temperature. The protein concentrations were varied by a serial dilution in the binding buffer that contains 20 mM Tris-HCl (pH 8.0), 200 mM NaCl, 10 mM DTT and excessive 5 μM of each tested compound. The fluorescence polarization measurements were carried out in triplets using 96-well black polystyrene plates on FlexStation 3. The data were processed and the Kd values were calculated in GraphPad Prism 5.

Circular dichroism (CD) temperature melting assays: The purified RORγ-LBD samples (0.1 mg/ml) were prepared in 10 mM phosphate (pH 7.0), 100 mM NaCl and incubated with 5 μM of each tested compound added. The CD-melting experiments were carried out on an AVIV circular dichroism spectrometer (Model 420) using a 10–80 °C gradient with 2 °C jumps and 2 min equilibration time. CD signals were recorded at 225 nM and the collected data points were fitted in KaleidaGraph for the calculation of melting temperatures (Tm) using the equations described in (Greenfield, 2006).

AlphaScreen® Assay: The binding efficiency of ligands to 6x HisTag hRORγ LBDs were determined by Perkin Elmer AlphaScreen® Histidine (PE 6760619C) or GST (PE 6760603C) detection kits. Assays were performed with 100 nM receptor LBD and 100 nM of biotinylated coactivator motif peptides in the presence of 10-20 μg/ml donor and acceptor beads in a buffer containing 50 mM MOPS pH 7.4, 50 mM NaF, 0.05 mM

CHAPS, and 0.1 mg/ml bovine serum albumin. The master mix was made by mixing all the components together and added to the chemicals to be tested. The reaction mixture was incubated for 90 min to equilibrate and read using a Wallac 2140 EnVision^{TM} multilabel plate reader in a 384 well plate format. The intensity of the photon count is directly proportional to the binding efficiency of the protein.

Statistical procedures: Unless otherwise stated data are shown as averages with standard deviations. Exclusion of samples with at least ±2 sd from the mean was only performed in statistical procedures that require two independent samples of equal size. Whenever possible, experiments were designed so that samples under comparison are of equal sizes. Basic comparisons were performed using non-randomized data analyzed by two-tailed unpaired Student's *t*-test with either equal or unequal variance as indicated. Variance assumptions of equality or inequality were based on Ftest of the sample. Aggregate data of multiple experiments were used to draw Q-Q plots to determine the normality of the distribution in the sample. The adequate sample size and power was estimated by using statistical power tables based on Cohen's d coefficient or Δ tables when Cohen's d coefficient was high. Calculations were performed using IBM SPSS statistics 20 package and the statistical functions of Excel.



Supplemental experimental procedures Figure 1: **A** Mass spectra of 4β,14α-dimethylcholesta-7,24-dien-3,30-diol (RRT*c* = 1.83). **B** Mass spectra of 4α-formyl-4β,14α-dimethylcholesta-7,24-dien-3-ol (RRT*c* = 1.95).

SEP Table I related to main text experimental procedures

Extended Data Table 1 Data collection and refinement statistics (**Molecular replacement**)

	RORα bound with 4ACD8	RORγ bound with 4ACD8
Data collection		
Space group	P $2_12_12_1$	P 6_322
Cell dimensions		
a, *b*, *c* (Å)	71.2, 80.3, 113.1	158.2, 158.2, 129.8
α, β, γ (°)	90, 90, 90	90, 90, 120
Resolution (Å)	50-1.9 (1.93-1.90) *	50-3.54 (3.57-3.54)
R_{sym} or R_{merge}	10.8 (82.5)	18.7 (97.2)
$I/\sigma I$	20.1 (1.8)	16.0 (2.8)
Completeness (%)	99.3 (97.6)	100.0 (100.0)
Redundancy	5.6 (3.3)	11.7 (12.3)
Refinement		
Resolution (Å)	46.24-1.90 (1.93-1.90)	47.11-3.54 (3.90-3.54)
No. reflections	51,607 (2,563)	12,041 (2,906)
R_{work}/ R_{free}	0.174/0.213 (0.254/0.291)	0.187/0.219 (0.206/0.228)
No. atoms		
Protein/peptide	4244	2075
Ligand/ion	76	32
Water	310	
B-factors		
Protein	33.1	73.5
Ligand/ion	51.7	82.6
Water	39.7	
R.m.s deviations		
Bond lengths (Å)	0.005	0.003
Bond angles (°)	0.907	0.755

*Highest resolution shell is shown in parenthesis.

Supplemental References

Aboushadi, N., Shackelford, J.E., Jessani, N., Gentile, A., and Krisans, S.K. (2000). Characterization of peroxisomal 3-hydroxy-3-methylglutaryl coenzyme A reductase in UT2 cells: sterol biosynthesis, phosphorylation, degradation, and statin inhibition. Biochemistry *39*, 237-247.

Adams, P.D., Grosse-Kunstleve, R.W., Hung, L.W., Ioerger, T.R., McCoy, A.J., Moriarty, N.W., Read, R.J., Sacchettini, J.C., Sauter, N.K., and Terwilliger, T.C. (2002). PHENIX: building new software for automated crystallographic structure determination. Acta Crystallogr D Biol Crystallogr *58*, 1948-1954.

Bhattacharya, B.S., Sweby, P.K., Minihane, A.M., Jackson, K.G., and Tindall, M.J. (2014). A mathematical model of the sterol regulatory element binding protein 2 cholesterol biosynthesis pathway. J Theor Biol *349*, 150-162.

Blais, C., Dauphin-Villemant, C., Kovganko, N., Girault, J.P., Descoins, C., Jr., and Lafont, R. (1996). Evidence for the involvement of 3-oxo-delta 4 intermediates in ecdysteroid biosynthesis. Biochem J *320 (Pt 2)*, 413-419.

Brunger, A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.S., Kuszewski, J., Nilges, M., Pannu, N.S., *et al.* (1998). Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr D Biol Crystallogr *54*, 905-921.

Chakravarthy, M.V., Lodhi, I.J., Yin, L., Malapaka, R.R., Xu, H.E., Turk, J., and Semenkovich, C.F. (2009). Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. Cell *138*, 476-488.

Ciofani, M., Madar, A., Galan, C., Sellars, M., Mace, K., Pauli, F., Agarwal, A., Huang, W., Parkurst, C.N., Muratet, M., *et al.* (2012). A validated regulatory network for Th17 cell specification. Cell *151*, 289-303.

Crabtree, S.R., Chu, W.L.A., and Mander, L.N. (1990). C-Acylation of enolates by methyl cyanoformate: an examination of site- and stereoselectivity. Synlett *1990*, 169-170.

Eberl, G., and Littman, D.R. (2004). Thymic origin of intestinal alphabeta T cells revealed by fate mapping of RORgammat+ cells. Science *305*, 248-251.

Edwards, P.A., Muroya, H., and Gould, R.G. (1972). In vivo demonstration of the circadian thythm of cholesterol biosynthesis in the liver and intestine of the rat. J Lipid Res *13*, 396-401.

Emsley, P., and Cowtan, K. (2004). Coot: model-building tools for molecular graphics. Acta Crystallogr D Biol Crystallogr *60*, 2126-2132.

Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem *226*, 497-509.

Gachotte, D., Barbuch, R., Gaylor, J., Nickel, E., and Bard, M. (1998). Characterization of the Saccharomyces cerevisiae ERG26 gene encoding the C-3 sterol dehydrogenase (C-4 decarboxylase) involved in sterol biosynthesis. Proc Natl Acad Sci U S A *95*, 13794-13799.

Goad, L.J., and Akihisa, T. (1997). Analysis of Sterols (London: Chapman & Hall).

Greenfield, N.J. (2006). Using circular dichroism collected as a function of temperature to determine the thermodynamics of protein unfolding and binding interactions. Nat Protoc *1*, 2527-2535.

Huh, J.R., Leung, M.W., Huang, P., Ryan, D.A., Krout, M.R., Malapaka, R.R., Chow, J., Manel, N., Ciofani, M., Kim, S.V., *et al.* (2011). Digoxin and its derivatives suppress TH17 cell differentiation by antagonizing RORgammat activity. Nature *472*, 486-490.
Huh, J.R., and Littman, D.R. (2012). Small molecule inhibitors of RORgammat: targeting Th17 cells and other applications. Eur J Immunol *42*, 2232-2237.
Ivanova, N., Dobrin, R., Lu, R., Kotenko, I., Levorse, J., DeCoste, C., Schafer, X., Lun, Y., and Lemischka, I.R. (2006). Dissecting self-renewal in stem cells with RNA interference. Nature *442*, 533-538.
Keber, R., Motaln, H., Wagner, K.D., Debeljak, N., Rassoulzadegan, M., Acimovic, J., Rozman, D., and Horvat, S. (2011). Mouse knockout of the cholesterogenic cytochrome P450 lanosterol 14alpha-demethylase (Cyp51) resembles Antley-Bixler syndrome. J Biol Chem *286*, 29086-29097.
Lee, P.P., Fitzpatrick, D.R., Beard, C., Jessup, H.K., Lehar, S., Makar, K.W., Perez-Melgosa, M., Sweetser, M.T., Schlissel, M.S., Nguyen, S., *et al.* (2001). A critical role for Dnmt1 and DNA methylation in T cell development, function, and survival. Immunity *15*, 763-774.
Mazur, Y., and Sondheimer, F. (1958). Synthesis of 4α-methyl-Δ7-steroids. The interrelationship of cholesterol, citrostadienol and lophenol. . J Am Chem Soc *80*, 6296-6299
McCoy, A.J., Grosse-Kunstleve, R.W., Adams, P.D., Winn, M.D., Storoni, L.C., and Read, R.J. (2007). Phaser crystallographic software. J Appl Crystallogr *40*, 658-674.
Medvedev, A., Yan, Z.H., Hirose, T., Giguere, V., and Jetten, A.M. (1996). Cloning of a cDNA encoding the murine orphan receptor RZR/ROR gamma and characterization of its response element. Gene *181*, 199-206.
Mialoundama, A.S., Jadid, N., Brunel, J., Di Pascoli, T., Heintz, D., Erhardt, M., Mutterer, J., Bergdoll, M., Ayoub, D., Van Dorsselaer, A., *et al.* (2013). Arabidopsis ERG28 tethers the sterol C4-demethylation complex to prevent accumulation of a biosynthetic intermediate that interferes with polar auxin transport. Plant Cell *25*, 4879-4893.
Miller, M.B., Haubrich, B.A., Wang, Q., Snell, W.J., and Nes, W.D. (2012). Evolutionarily conserved Delta(25(27))-olefin ergosterol biosynthesis pathway in the alga Chlamydomonas reinhardtii. J Lipid Res *53*, 1636-1645.
Minor, W., Cymborowski, M., Otwinowski, Z., and Chruszcz, M. (2006). HKL-3000: the integration of data reduction and structure solution--from diffraction images to an initial model in minutes. Acta Crystallogr D Biol Crystallogr *62*, 859-866.
Mo, C., Valachovic, M., Randall, S.K., Nickels, J.T., and Bard, M. (2002). Protein-protein interactions among C-4 demethylation enzymes involved in yeast sterol biosynthesis. Proc Natl Acad Sci U S A *99*, 9739-9744.
Nes, W.D., Zhou, W., Ganapathy, K., Liu, J., Vatsyayan, R., Chamala, S., Hernandez, K., and Miranda, M. (2009). Sterol 24-C-methyltransferase: an enzymatic target for the disruption of ergosterol biosynthesis and homeostasis in Cryptococcus neoformans. Arch Biochem Biophys *481*, 210-218.
Pelham, H.R. (1982). A regulatory upstream promoter element in the Drosophila hsp 70 heat-shock gene. Cell *30*, 517-528.

Quehenberger, O., Armando, A.M., Brown, A.H., Milne, S.B., Myers, D.S., Merrill, A.H., Bandyopadhyay, S., Jones, K.N., Kelly, S., Shaner, R.L., *et al.* (2010). Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res *51*, 3299-3305.
Rahier, A., Darnet, S., Bouvier, F., Camara, B., and Bard, M. (2006). Molecular and enzymatic characterizations of novel bifunctional 3beta-hydroxysteroid dehydrogenases/C-4 decarboxylases from Arabidopsis thaliana. J Biol Chem *281*, 27264-27277.
Rahimtula, A.D., and Gaylor, J.L. (1972). Partial purification of a microsomal sterol 4 -carboxylic acid decarboxylase. J Biol Chem *247*, 9-15.
Rondet, S., Taton, M., and Rahier, A. (1999). Identification, characterization, and partial purification of 4 alpha-carboxysterol-C3-dehydrogenase/ C4-decarboxylase from Zea mays. Arch Biochem Biophys *366*, 249-260.
Sadowski, I., and Ptashne, M. (1989). A vector for expressing GAL4(1-147) fusions in mammalian cells. Nucleic Acids Res *17*, 7539.
Shapiro, D.J., and Rodwell, V.W. (1971). Regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol synthesis. J Biol Chem *246*, 3210-3216.
Simmons, E.M., and Hartwig, J.F. (2012). Catalytic functionalization of unactivated primary C-H bonds directed by an alcohol. Nature *483*, 70-73.
Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., *et al.* (2011). A conditional knockout resource for the genome-wide study of mouse gene function. Nature *474*, 337-342.
Smith, C.A., Want, E.J., O'Maille, G., Abagyan, R., and Siuzdak, G. (2006). XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem *78*, 779-787.
Sun, Z., Unutmaz, D., Zou, Y.R., Sunshine, M.J., Pierani, A., Brenner-Morton, S., Mebius, R.E., and Littman, D.R. (2000). Requirement for RORgamma in thymocyte survival and lymphoid organ development. Science *288*, 2369-2373.
Tanaka, R.D., Edwards, P.A., Lan, S.F., Knoppel, E.M., and Fogelman, A.M. (1982). Purification of 3-hydroxy-3-methylglutaryl coenzyme A reductase from human liver. J Lipid Res *23*, 523-530.
Trzaskos, J.M., Favata, M.F., Fischer, R.T., and Stam, S.H. (1987). In situ accumulation of 3 beta-hydroxylanost-8-en-32-aldehyde in hepatocyte cultures. A putative regulator of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity. J Biol Chem *262*, 12261-12268.
van de Pavert, S.A., Olivier, B.J., Goverse, G., Vondenhoff, M.F., Greuter, M., Beke, P., Kusser, K., Hopken, U.E., Lipp, M., Niederreither, K., *et al.* (2009). Chemokine CXCL13 is essential for lymph node initiation and is induced by retinoic acid and neuronal stimulation. Nat Immunol *10*, 1193-1199.
van den Elzen, P., Garg, S., Leon, L., Brigl, M., Leadbetter, E.A., Gumperz, J.E., Dascher, C.C., Cheng, T.Y., Sacks, F.M., Illarionov, P.A., *et al.* (2005). Apolipoprotein-mediated pathways of lipid antigen presentation. Nature *437*, 906-910.
Xu, S., Norton, R.A., Crumley, F.G., and Nes, W.D. (1988). Comparison of the chromatographic properties of sterols, select additional steroids and triterpenoids: Gravity-flow column liquid chromatography, thin-layer chromatography, gas-liquid

chromatography and high-performance liquid chromatography. . JChromatog A *452*, 377-398.